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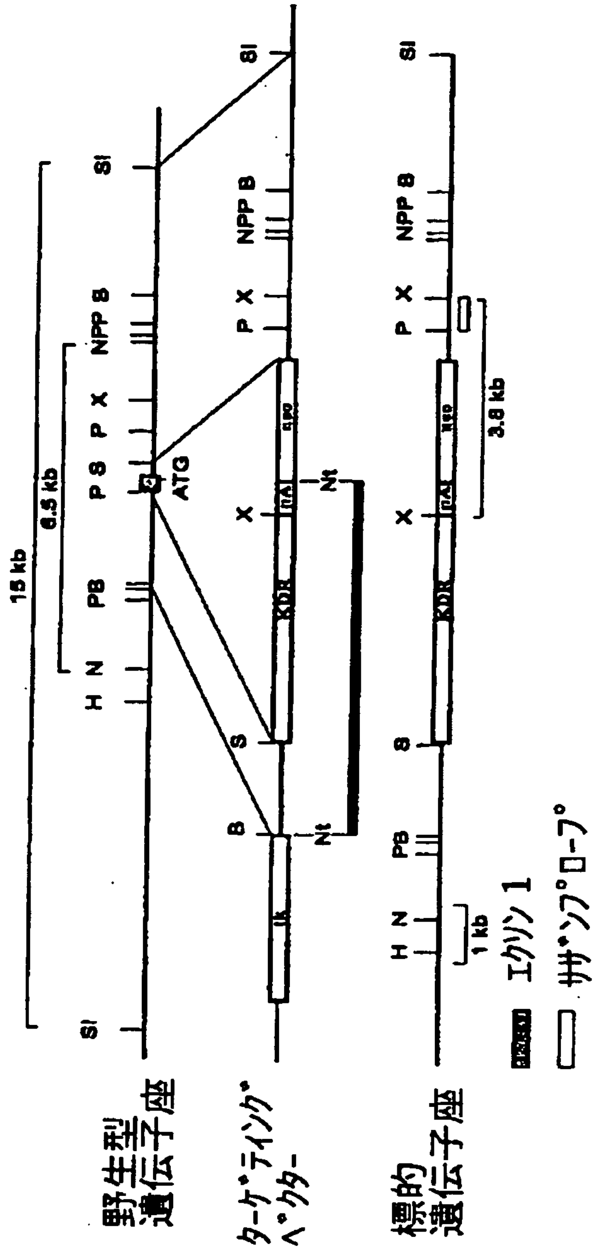
【図1】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標的遺伝子座
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サザンプローブ
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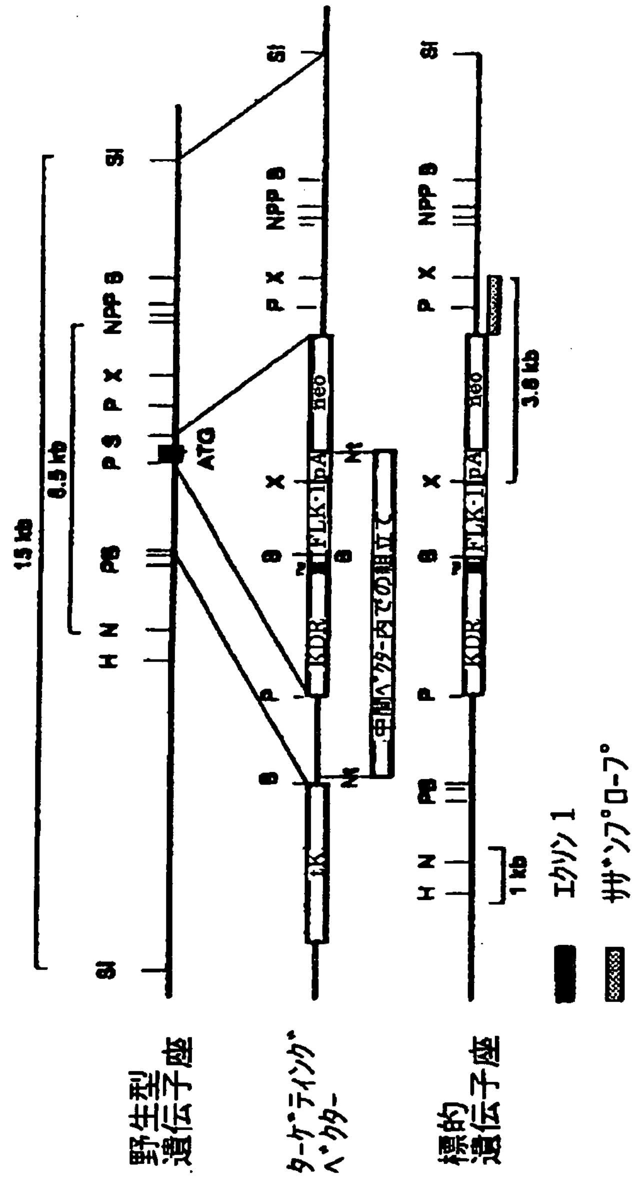
6.5 kb
3.8 kb
1 kb
ATG
KDR
Nt
Sl
H N
PB
P S
P X
NPP B
S
B
X

【図2】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ベクター
標的
遺伝子座
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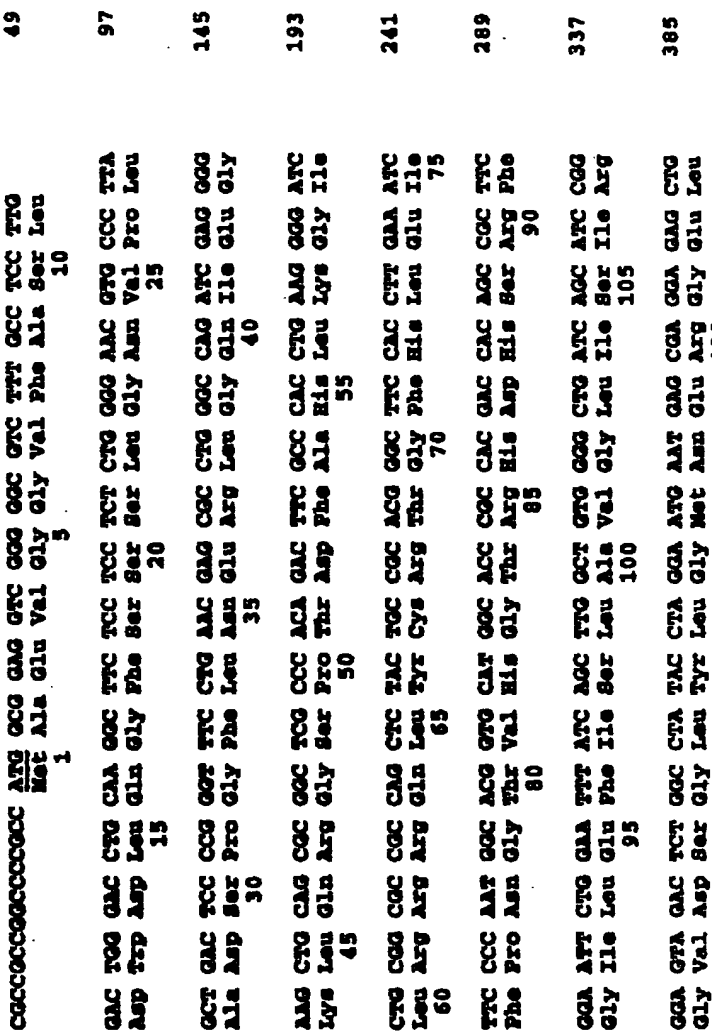
6.5 kb
3.8 kb
1 kb
ATG
KDR
FLK-1pA
neo
tK
中間ベクター内での組立て
エクソン1
サザンプローブ

【図1A】

FIG.1A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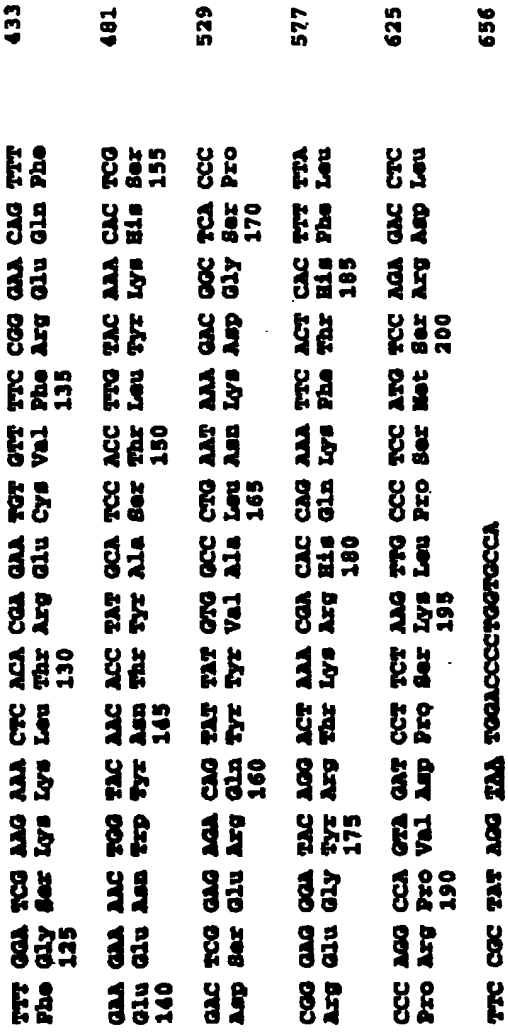
1 5 10
GAC TGG GAC CTG CAA GGC TTC TCC TCC TCT CTG GGG AAC GTG CCC TTA 97
Asp Trp Asp Leu Gln Gly Phe Ser Ser Ser Leu Gly Asn Val Pro Leu
15 20 25
GCT GAC TCC CCG GGT TTC CTG AAC GAG CGC CTG GGC CAG ATC GAG GGG 145
Ala Asp Ser Pro Gly Phe Leu Asn Glu Arg Leu Gly Gln Ile Glu Gly
30 35 40
AAG CTG CAG CGC GGC TCG CCC ACA GAC TTC GCC CAC CTG AAG GGG ATC 193
Lys Leu Gln Arg Gly Ser Pro Thr Asp Phe Ala His Leu Lys Gly Ile
45 50 55
CTG CGG CGC CGC CAG CTC TAC TGC CGC ACG GGC TTC CAC CTT GAA ATC 241
Leu Arg Arg Arg Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Glu Ile
60 65 70 75
TTC CCC AAT GGC ACG GTG CAT GGC ACC CGC CAC GAC CAC AGC CGC TTC 289
Phe Pro Asn Gly Thr Val His Gly Thr Arg His Asp His Ser Arg Phe
80 85 90
GGA ATT CTG GAA TTT ATC AGC TTG GCT GTG GGG CTG ATC AGC ATC CGG 337
Gly Ile Leu Glu Phe Ile Ser Leu Ala Val Gly Leu Ile Ser Ile Arg
95 100 105
GGA GTA GAC TCT GGC CTA TAC CTA GGA ATG AAT GAG CGA GGA GAG CTG 385
Gly Val Asp Ser Gly Leu Tyr Leu Gly Met Asn Glu Arg Gly Glu Leu
110 115 120

【図1B】

FIG.1B


433
481
529
577
625
656
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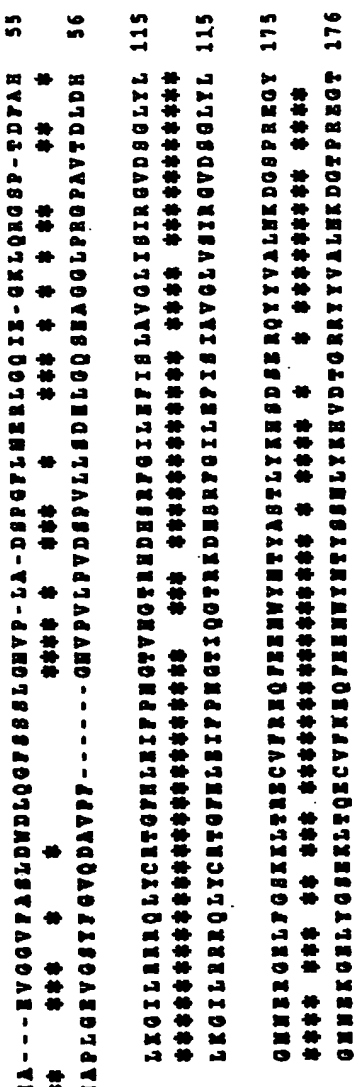
Phe Gly Ser Lys Lys Leu Thr Arg Glu Cys Val Phe Arg Glu Gln Phe
125 130 135
GAA GAA AAC TGG TAC AAC ACC TAT GCA TCC ACC TTG TAC AAA GAC GGC TCA CCC
Glu Glu Asn Trp Tyr Asn Thr Tyr Ala Ser Thr Leu Tyr Lys Asp Gly Ser Pro
140 145 150 155 160 165 170
GAC TCG GAG AGA CAG TAT TAT GTG GCC CTG AAT AAA GAC GGC TCA CCC
Asp Ser Glu Arg Gln Tyr Tyr Val Ala Leu Asn Lys Asp Gly Ser Pro
175 180 185 190 195 200
CGG GAG GGA TAC AGG ACT AAA CGA CAC CAG AAA TTC ACT GAA TTT TTA
Arg Glu Gly Tyr Arg Thr Lys Arg His Gln Lys Phe Thr Glu Phe Leu
CCC AGG CCA GTA GAT CCT TCT AAG TTG CCC TCC ATG TCC AGA GAC CTC
Pro Arg Pro Val Asp Pro Ser Lys Leu Pro Ser Met Ser Arg Asp Leu
TTC CGC TAT AGG TAA TGGACCCCTGGTGCCA
Phe Arg Tyr Arg

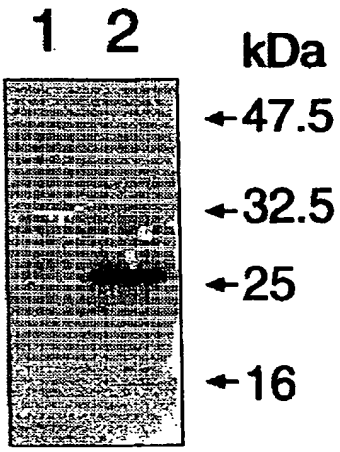
【図2】

FIG.2



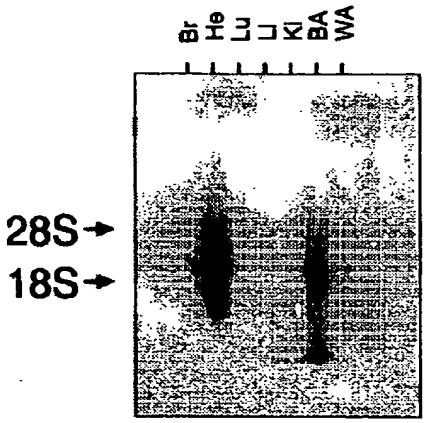
【図3】

FIG.3



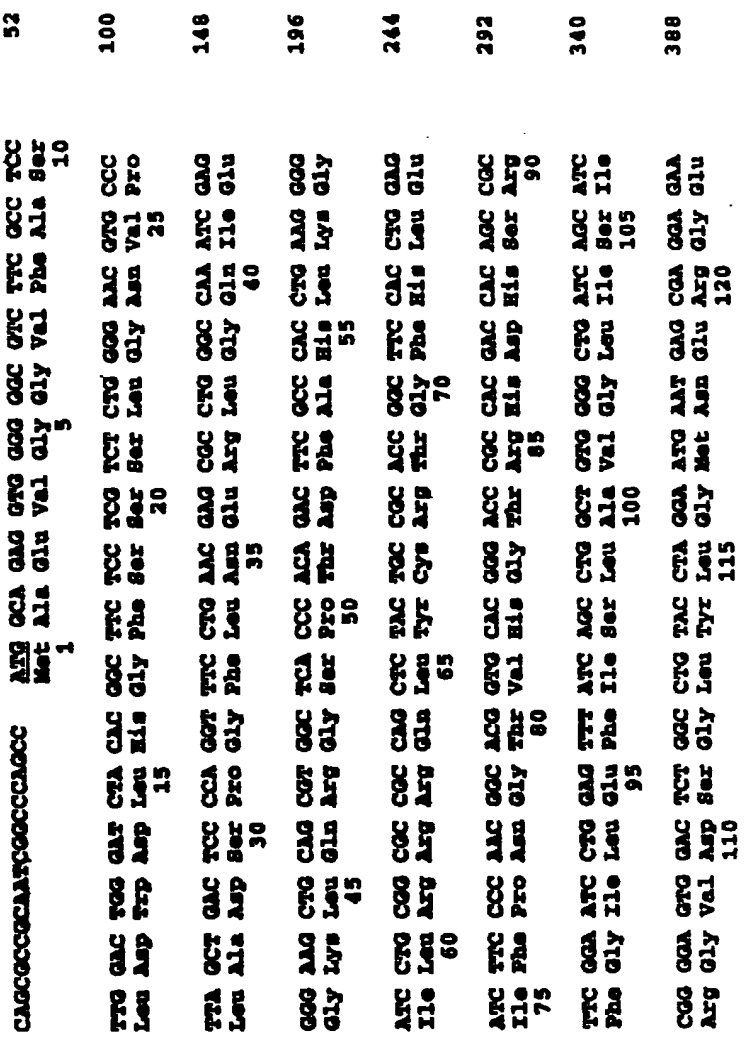
【図4】

FIG.4



【図6A】

FIG.6A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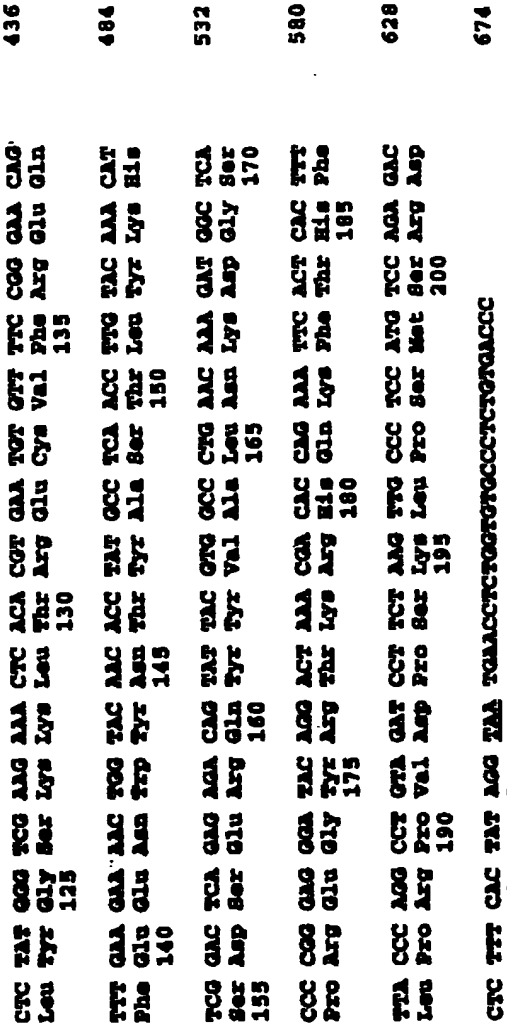
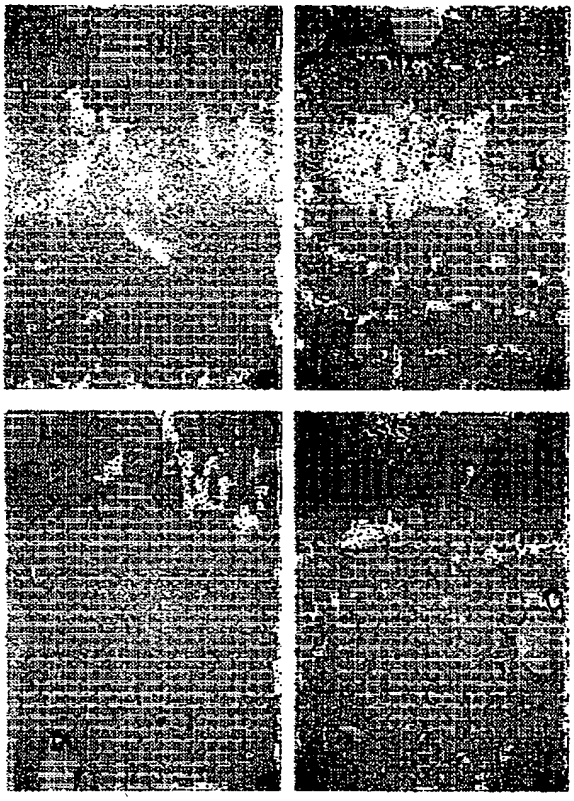
Met Ala Glu Val Gly Gly Val Phe Ala Ser
1 5 10
52
TTG GAC TGG GAT CTA CAC GGC TTC TCC TCG TCT CTG GGG AAC GTG CCC
Leu Asp Trp Asp Leu His Gly Phe Ser Ser Ser Leu Gly Asn Val Pro
15 20 25
100
TTA GCT GAC TCC CCA GGT TTC CTG AAC GAG CGC CTG GGC CAA ATC GAG
Leu Ala Asp Ser Pro Gly Phe Leu Asn Glu Arg Leu Gly Gln Ile Glu
30 35 40
148
GGG AAG CTG CAG CGT GGC TCA CCC ACA GAC TTC GCC CAC CTG AAG GGG
Gly Lys Leu Gln Arg Gly Ser Pro Thr Asp Phe Ala His Leu Lys Gly
45 50 55
196
ATC CTG CGG CGC CGC CAG CTC TAC TGC CGC ACC GGC TTC CAC CTG GAG
Ile Leu Arg Arg Arg Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Glu
60 65 70
244
ATC TTC CCC AAC GGC ACG GTG CAC GGG ACC CGC CAC GAC CAC AGC CGC
Ile Phe Pro Asn Gly Thr Val His Gly Thr Arg His Asp His Ser Arg
75 80 85 90
292
TTC GCA ATC CTG GAG TTT ATC AGC CTG GCT GTG GGG CTG ATC AGC ATC
Phe Gly Ile Leu Glu Phe Ile Ser Leu Ala Val Gly Leu Ile Ser Ile
95 100 105
340
CGG GCA GTG GAC TCT GGC CTG TAC CTA GGA ATG AAT GAG CGA GGA GAA
Arg Gly Val Asp Ser Gly Leu Tyr Leu Gly Met Asn Glu Arg Gly Glu
110 115 120
388

【図6B】

FIG.6B



【図7】



【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/17919

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/50, C07K 16/22, A61K 38/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Biochem Biophys Res Commun, Volume 243, No 1, February 1998, Miyake A. et al, "Structure and expression of a novel member, FGF-16, on the fibroblast growth factor family" page 148 - page 152 --	1-3,5-36
A	EP 0503297 A1 (TAKEDA CHEMICAL INDUSTRIES, LTD.), 16 Sept 1992 (16.09.92), SEQ ID No 5 --	1-3,5-36
A	EP 0619370 A1 (AMGEN INC.), 12 October 1994 (12.10.94), page 2, line 41 - line 43 --	1-3,5-36

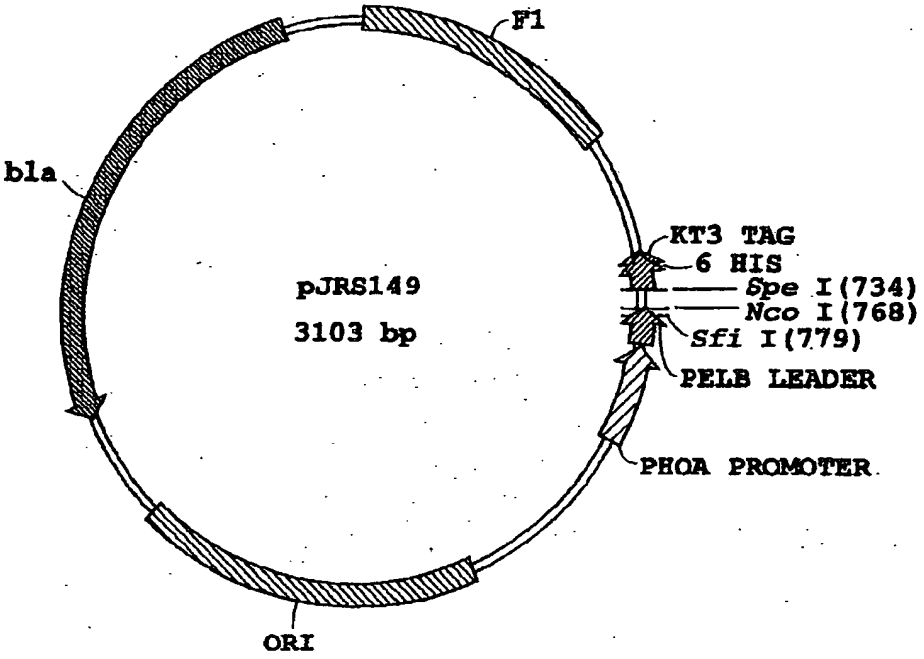
[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

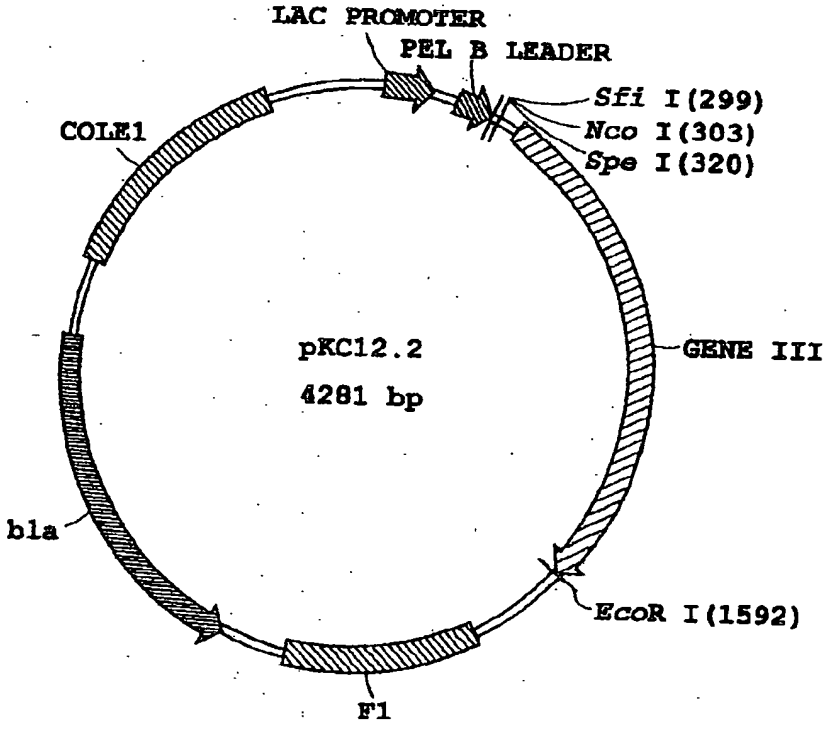
Date of the actual completion of the international search	Date of mailing of the international search report
8 December 1998	15. 01. 99
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Patrick Andersson

Form PCT/ISA/210 (second sheet) (July 1992)

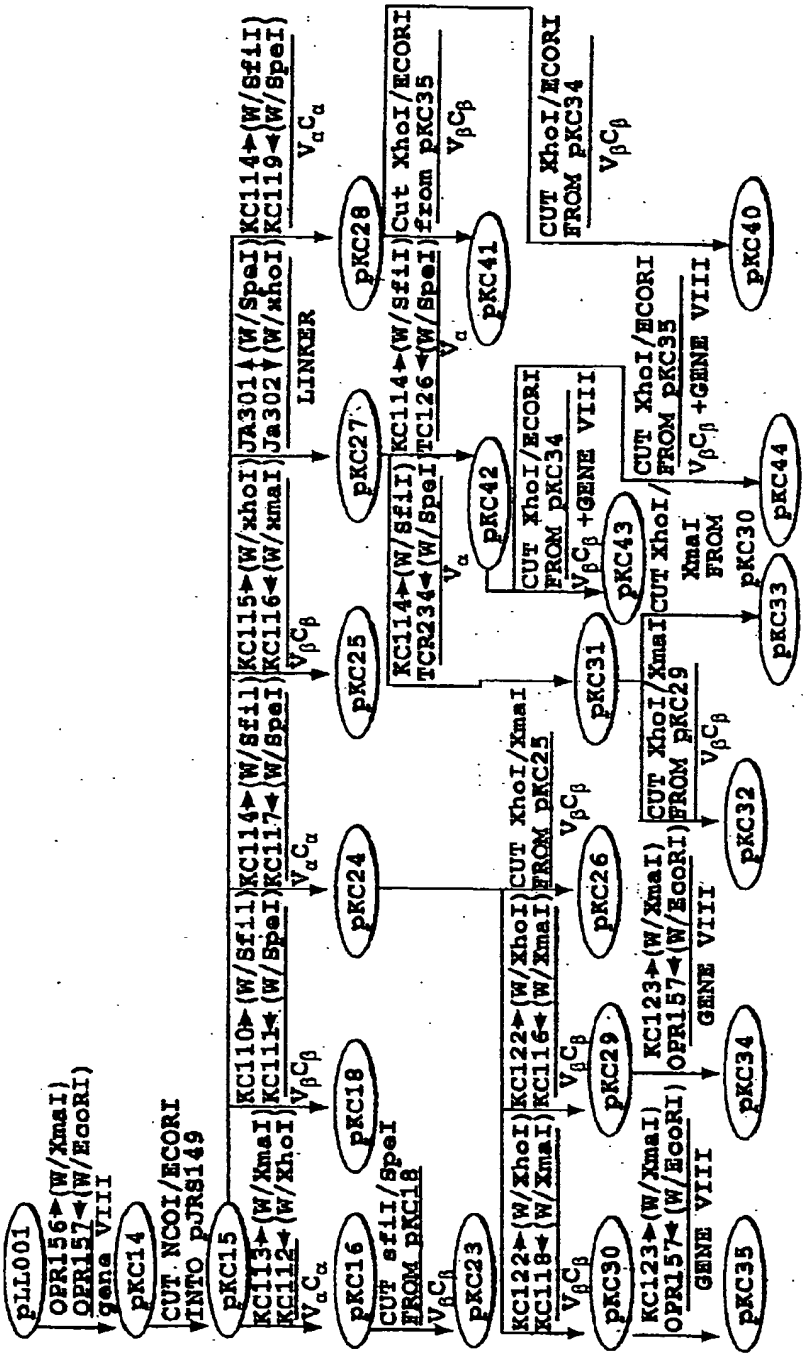
【図1】


F1
bla
KT3 TAG
6 HIS
Spe I(734)
Nco I(768)
Sfi I(779)
PELB LEADER
pJRS149
3103 bp
PHOA PROMOTER
ORI

【図2】


LAC PROMOTER
PEL B LEADER
Sfi I(299)
Nco I(303)
Spe I(320)
COLE1
pKC12.2
4281 bp
GENE III
bla
EcoR I(1592)
F1

【図3】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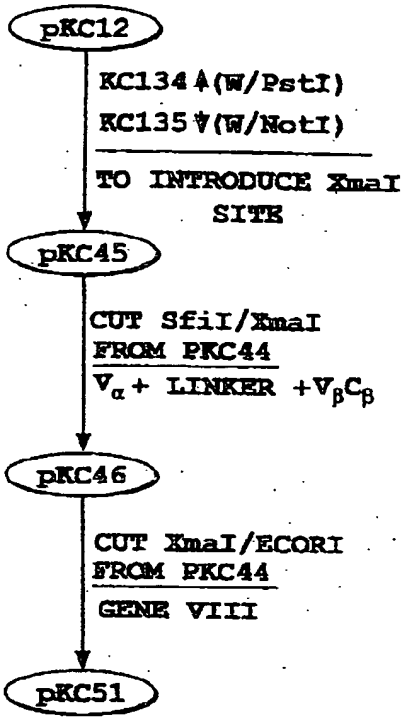
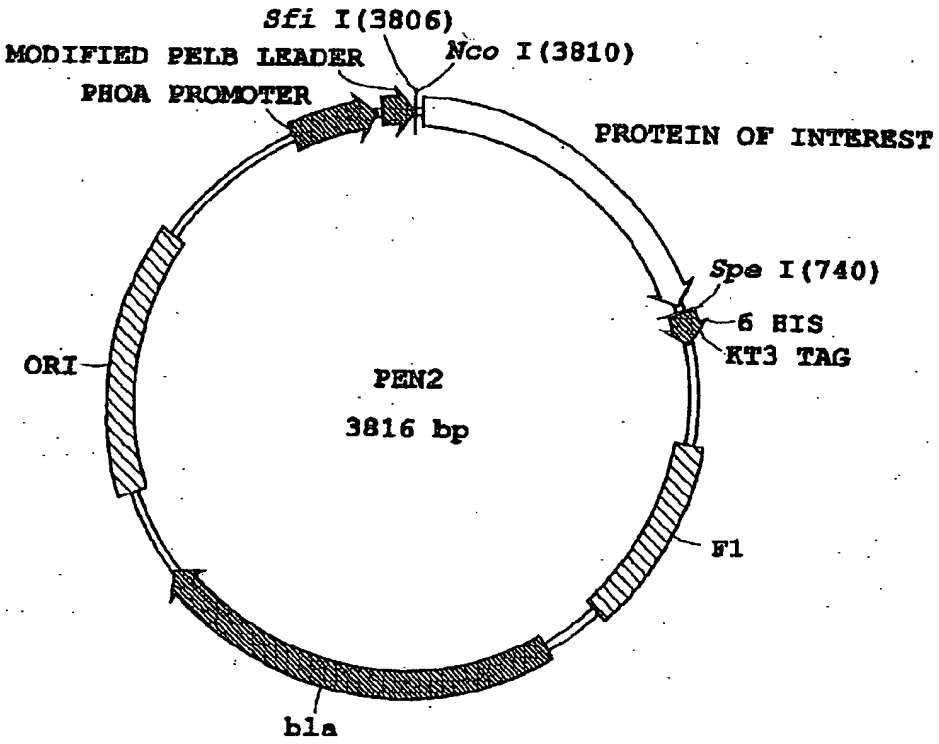
OPR156→(W/XmaI)
OPR157←(W/EcoRI)
gene VIII
pKC14
CUT NCOI/ECORI
INTO pJR8149
pKC15
KC110→(W/SfiI)
KC111←(W/XhoI)
KC113→(W/XmaI)
KC112←(W/SpeI)
pKC16
CUT sfiI/SpeI
FROM pKC18
pKC18
pKC23
KC114→(W/SfiI)
KC117←(W/SpeI)
pKC24
KC122→(W/XhoI)
KC118←(W/XmaI)
pKC30
KC123→(W/XmaI)
OPR157←(W/EcoRI)
GENE VIII
pKC35
KC122→(W/XhoI)
KC116←(W/XmaI)
pKC29
KC123→(W/XmaI)
OPR157←(W/EcoRI)
GENE VIII
pKC34
CUT XhoI/XmaI
FROM pKC25
pKC26
pKC32
KC115→(W/XhoI)
KC116←(W/XmaI)
pKC25
pKC27
KC114→(W/SfiI)
TC234←(W/SpeI)
pKC42
CUT XhoI/ECORI
FROM pKC34
pKC43
CUT XhoI/
XmaI
FROM
pKC30
pKC33
JA301→(W/SpeI)
Ja302←(W/XhoI)
LINKER
pKC28
KC114→(W/SfiI)
TC126←(W/SpeI)
pKC41
Cut XhoI/ECORI
from pKC35
CUT XhoI/ECORI
FROM pKC34
CUT XhoI/ECORI
FROM pKC35
pKC40
pKC44

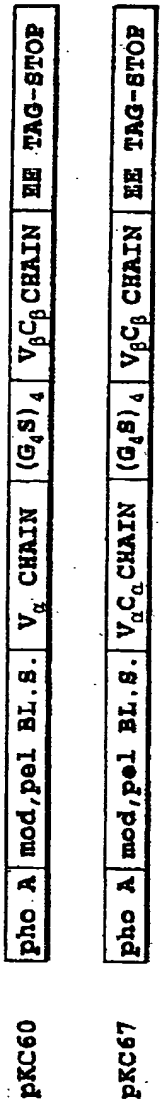
【図4】


pKC12
KC134↑(W/PstI)
KC135↓(W/NotI)
TO INTRODUCE XmaI SITE
pKC45
CUT SfiI/XmaI FROM PKC44
V_α + LINKER +$V_\beta C_\beta$
pKC46
CUT XmaI/ECORI FROM PKC44
GENE VIII
pKC51

【図5】


Sfi I(3806)
MODIFIED PELB LEADER
PHOA PROMOTER
Nco I(3810)
PROTEIN OF INTEREST
Spe I(740)
6 HIS
KT3 TAG
ORI
PEN2
3816 bp
F1
bla

【図6】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pho A
mod,pel BL.S.
V_α CHAIN
$(G_4S)_4$
$V_\beta C_\beta$ CHAIN
EE TAG-STOP
pKC67
pho A
mod,pel BL.S.
$V_\alpha C_\alpha$ CHAIN
$(G_4S)_4$
$V_\beta C_\beta$ CHAIN
EE TAG-STOP

【図7】

Primer	Seq Id No	Sequences (5' to 3')
KC100	001	CGG CCA TGG CCC AGC TGC AGA CTA GTG C
KC101	002	GGC CGC ACT AGT CTG CAG CTG GGC CAT GGC CGG CT
KC110	003	CTC GCG GCC CAG CCG GCC ATG GCC GAG GCT GCA GTC ACC CAA AGC
KC111	004	CTT CCT CAC TAG TAC AGT CTG CTC GGC CCC AG
KC112	005	GAT GGC CTC GAG GAG CAG GTG GAG CAG CTT
KC113	006	GAC TAG CCC GGG ACA GGG AAC GTC TGA ACT GGG
KC114	007	CTC GCG GCC CAG CCG GCC ATG GCC GAG CAG GTG GAG CAG CTT CCT
KC115	008	CTC GCG CTC GAG GAG GCT GCA GTC ACC CAA AGC
KC116	009	CTC GCG CCC GGG ACA GTC TGC TCG GCC CCA GGC
KC117	010	CTC GCG ACT AGT ACA GGG AAC GTC TGA ACT GGG
KC118	011	CTC GCG CCC GGG GTC TGC TCG GCC CCA GGC
KC119	012	CTC GCG ACT AGT GGG AAC GTC TGA ACT GGG
KC120	013	CTC GCG ACT AGT GTC TGC TCG GCC CCA GGC
KC121	014	CTC GCG CCC GGG GGG AAC GTC TGA ACT GGG
KC122	015	CTC GCG CTC GAG CGA GGC TGC AGT CAC CCA AAG C
KC123	016	GGG GGG CCC GGG GCT GAG GGT GAC GAT CCC GCA AAA G

【図8】

Primer	Seq Id No	Sequences (5' to 3')
KC124	017	CTA GTC TGG TGG CGG TGG CAG CGG CGG TGG TGG TTC CGG TGG CGG CGG TTC TGG CGG TGG CGG TTC C
KC125	018	TCG AGG AAC CGC CAC CGC CAG AAC CGC CGC CAC CGG AAC CAC CAC CGC CGC TGC CAC CGC CAC CAG A
KC126	019	GTG CTC ACT AGT GTT TGG CTC TAC AGT GAG TTT GGT G
KC127	020	GAT GGC TCG AGT GAG CAG GTG GAG CAG CTT CCT
KC128	021	CTA GTC CCC GGG TAC AAC TGT GAG TCT GGT TCC
KC129	022	CTC GAG ACT AGT TAC AAC TGT GAG TCT GGT TCC
KC130	023	CGG CCG AGG AAG AAG AGT ACA TCC CGA TGG ATC
KC131	024	GGG CCA TCC ATC GGG ATG TAC TCT TCT TCC TCG GCC GGC T
KC132	025	CCG GGG AGG AAG AAG AGT ACA TCC CGA TGG ATT GAG
KC133	026	AAT TCT CAA TCC ATC GGG ATG TAC TCT TCT TCC TCC
KC134	027	GCC CGG GAC TAG TGC
KC135	028	GGC CGC ACT AGT CCC GGG CTG CA
KC136	029	CTA GTC CCC GGG TCA TCA AGC GGC GCC TTC CAT CGG CAT GTA CTC TTC TTC CTC TAC AAC TGT GAG TCT GGT TCC
KC137	030	CTA GTC CCC GGG TCA TCA AGC GGC GCC TTC CAT CGG CAT GTA CTC TTC TTC CTC GTC TGC TCG GCC CCA GGC

【図9】

Primer	Seq Id No	Sequences (5' to 3')
KC138	031	CTA GTC CCC GGG TAC AAC TGT GAG TCT GGT TCC
KC139	032	CCG GGG AGG AAG AAG AGT ACA TGC CGA TGG AAG GCG CCG CTT AGC
KC140	033	CCT CCT TCT TCT CAT GTA CGG CTA CCT TCC GCG GCG AAT CGG GCC
KC141	034	GAT CAG CCC GGG GAG GCT GCA GTC ACC CAA AGC
KC142	035	CTA GTC CCC GGG ACA GTC TGC TCG GCC CCA CCG
KC143	036	CCG GGG AGG AAG AAG AGT ACA TGC CGA TGG AAG GCG CCG CTC
KC144	037	CCT CCT TCT TCT CAT GTA CGG CTA CCT TCC GCG GCG AGG GCC
KC145	038	CGC CGC TCA CCA TCA CCA TCA TCA CTG ATG AC
KC146	039	GGC GAG TGG TAG TGG TAG TAG TGA CTA CTG GGC C
KC147	040	GAT CAG GGC GCC GCT ACT GTT GAA AGT TGT TTA
KC148	041	CTG ATC GGA TCC TCA TTA AAG CCA GAA TGG AAA
KC149	042	CCG GGC TAA GCG GCG CCT TCC ATC GGC ATG TAC TCT TCT TCC TCC
KC150	043	CCG GGA GCG GCG CCT TCC ATC GGC ATG TAC TCT TCT TCC TCC
KC151	044	CCG GGT CAT CAG TGA TGA TGG TGA TGG TGA GCG G
KC152	045	GCT CGA GCT TAC TCC

【図10】

Primer	Seq Id No	Sequences (5' to 3')
KC153	046	CGC TCA TTA GGC GG
KC154	047	GTG TAC TTC TGT GCC
KC155	048	CTG TGA GTC TGG TTC
KC156	049	GCA GGT TCT GGG TTC
KC157	050	CAT TTA CTA ACG TCT GG
KC158	051	CGC CTG GTA CTG AGC
KC159	052	CCT CAA CCT CCT GTC
KC160	053	CTT ATT CCG TGG TGT C
KC161	054	CCA CCC TCA GAA CCG
KC162	055	GAA TTT ACC GTT CCA G
KC163	056	CTT TAG CGT CAG ACT G
KC164	057	GAA ACG CAA AGA CAC C
OPR156	058	GGG GGG CCC GGG CTG CTG AGG GTG ACG ATC CCG CAA AAG
OPR157	059	GGG GGG GAA TTC TAT TAG CTT GCT TTC GAG GTG AAT TTC
JWTCR222	060	GAG CAC GGC CCA GCC GGC CAT GGC CGA GGC TGC AGT CAC CC
JWTCR221	061	GAG CAC GAG ACT AGT AGC ACG AAC AAC ACG GTC GTC GAT CGG TTC CGG CGG GTT TGG CTC TAC AGT GAG
JWTCR220	062	GAT CCC TCC TGG ACA CGC AGG ATG GAA GGA AGC TGC TCC ACC TGC TCA GCA CGA ACA ACA CGG TCG TCG ATC GGT TCC GGC GGG GC
JWTCR219	063	CAT GGC CCC GCC GGA ACC GAT CGA CGA CCG TGT TGT TCG TGC TGA GCA GGT GGA GCA GCT TCC TTC CAT CCT GCG TGT CCA GGA GG

【図11】

Primer	Seq Id No	Sequences (5' to 3')
JWTCR218	064	GAG GTG GAA TTC TAT TAA GAC TCC TTA TTA CGC AGT ATG
JWTCR217B	065	GAG GAG GTG GTG ACT AGT AGC AGG TTC TGG TGG GTT CTG GAT GTT TGG CTC TAC AGT GAG
JWTCR217	066	GAG GAG GTG GTG ACT AGA AGC AGG TTC TGG GTT CTG GAT GTT TGG CTC TAC AGT GAG
JWTCR216	067	GAG GTG GAA TTC TAT TAG TGA TGA TGG TGA TGG TGA GAC TCC TTA TTA CGC
JWTCR215	068	GAG GTG CCC GGG ACT GTT GAA AGT TGT TTA GC
JWTCR214	069	GAG GTG GAA TTC TAT TAG TGA TGA TGG TGA TGG TGG CTT GCT TTC GAG G
JWTCR213	070	GAG GTG GAA TTC TAT TAG CTT GCT TTC GAG G
JWTCR212	071	GAG GTG CCC GGG GCT GAG GGT GAC GAT CCC G
JWTCR211	072	AAT TCT CAT CAG TGA TGA TGG TGA TGG TGC
JWTCR210	073	CCG GGC ACC ATC ACC ATC ATC ACT GAT GAG
JWTCR209	074	GTG GAG CCC GGG TTC CAT CGG CAT GTA CTC TTC TTC CTC TAC AAC TGT GAG TCT GG
JWTCR208	075	GAG GTG GAA TTC TCA CCC GGG TTC CAT CGG CAT GTA CTC TTC TTC CTC GTC TGC TCG GCC CCA G
JWTCR207	076	GAG GTG CTG CAG GTT CCA TCG GCA TGT ACT CTT CTT CCT CGT CTA GAC GGC CCC AGG CCT C
JWTCR206	077	GTG GAG CTG CAG GGT CTA GAC GGC CCC AGG CCT C
JWTCR204	078	GTG GAG CTG CAG GTG ATC CAC CCC CTC CAG ATC CAC CCC CTC CGT CTG CTC GGC CCC AG
JWTCR202	079	GTG GAG AAG CTT TGC CGA GCA GGT GGA GCA GC

【図12】

Primer	Seq Id No	Sequences (5' to 3')
JWTCR200A	080	GGG GGG GAG GTG CTC GAG CGA GGC AGC AGT CAC C
JWTCR23A	081	GAG CCC ACT AGT TTG GCT CTA CAG TGA GTT TGG TG
JWTCR1	082	CTA GAC CAG CAA ATC TGC ACC CAC AGA ATC CCT AGG ACA GCT CCC AGG TTC CTC TGC ATG GTG GA
JWTCR2	083	AGC TTC CAC CAT GCA GAG GAA CCT GGG AGC TGT CCT AGG GAT TCT GTG GGT GCA GAT TTG CTG GT
JWTCR3	084	GAT CGG TCT AGA GGT GAG CAG GTG GAG CAG CTT CC
JWTCR4	085	GCC TGG AGA CTC AGC CAT G
JWTCR5	086	GAA GTA CAT GGC TGA GTC TCC
JWTCR6	087	GAT GAA CGT TCC AGA TTC CAT GG
JWTCR7	088	CCC AAA TCA ATG TGC CGA AAA C
JWTCR8	089	CTA GAA CAC AGG AGA CTG GAG AGC ACG AAG AAG AGC CTG GAG CCC ATG GTG GA
JWTCR9	090	GCT CTC CTT GTA GGC CTG AG
JWTCR10	091	GTA CTT CTG TGC CAG CGG TG
JWTCR11	092	GAG CAA TTA TAG CTA CTG CCT G
JWTCR12	093	GGT CTG GAG GCC TTG TAT CC
JWTCR13	094	AGC TTC CAC CAT GGG CTC CAG GCT CTT CTT CGT GCT CTC CAG TCT CCT GTG TT
JA301	095	TCG AGG AAC CGC CAC CGC CAG AAC CGC CGC CAC CGG AAC CAC CAC CGC CGC TGC CAC CGC CAC CA

【図13】

Primer	Seq Id No	
JA302	96	CTA GTG GTG GCG GTG GCA GCG GCG GTG GTG GTT CCG GTG GCG GCG GTT CTG GCG GTG GCG GTT CC
KOZAK CONSENSUS		
	97	CCACCATG
	98	Glu Glu Glu Glu Tyr Met Pro Met Glu 1 5
	99	ATG AAA TAC CTG CTG CCG ACC GCA GCT GCT GGT CTG CTG CTG GCT GGC GGC CCA AGC CGA TGG CC
	100	MKY LLP TAA AAL LLL AAQ PAM

【図14】

PRIMERS TO AMPLIFY HUMAN TCR V_β LIBRARIES

TCR-Vβ FRONT:		SEQ. ID NO.
5'-GCCGGCCATGGCCRGTGCTGTCRTCTCTC	Vβ 2,4	101
5'-GCCGGCCATGGCCGAMRCCMARGTSACCC	10,14	102
5'-GCCGGCCATGGCCCGATGTGAAAGTAACCC	3	103
5'-GCCGGCCATGGCCGAMRCWGMCRTYTMCC	11,9	104
5'-GCCGGCCATGGCCARKGCTGGDGTCACTC	13,5-1	105
5'-GCCGGCCATGGCCAATGCCGGCGTCATGC	18	106
5'-GCCGGCCATGGCCGRTRCTGGAGTCTCCC	6	107
5'-GCCGGCCATGGCCGATGCTRGARTCACCC	8-3,12	108
5'-GCCGGCCATGGCCGATTCTGGAGTCACAC	1	109
5'-GCCGGCCATGGCCGAYGCTGGWGTTATCC	8-1,2,4	110
5'-GCCGGCCATGGCCGATGCTGRYRTTAYCC	8-5,15	111
5'-GCCGGCCATGGCCGAAGCTGGAGTTACTCAG	16	112
5'-GCCGGCCATGGCCGATGCTACTATTCATCAATGGCC[a]	20	113

TCR-V^β BACK:

MIXTURE OF:

5'-GCTGCCACCGCCACCCACCTTGTTCAGGTCC 114

5'-GCTGCCACCGCCACCCACGTTTTCAGGTCC 115

NcoI

[a]TWO CODONS WERE ADDED, BECAUSE FULL LENGHT V^β 20 cDNA WAS NOT AVAILABLE.

【図15】

PRIMERS TO AMPLIFY HUMAN TCR V_α LIBRARIES

TCR-Vα FRONT:		SEQ. ID NO.
5'-GGAGGCGGCGGTTCTGCTAAGAGACCACMCAGCC	Vα16,4	116
5'-GGAGGCGGCGGTTCTCAGAAGRTAACTCAARCSC	12,6	117
5'-GGAGGCGGCGGTTCTCAGTCKGTGASCCAGC	1,19	118
5'-GGAGGCGGCGGTTCTCAGAGAGTGACTCAGCC	9	119
5'-GGAGGCGGCGGTTCTCWGMASGTGRAACAARGTCC	13,14	120
5'-GGAGGCGGCGGTTCTCAGCAAGTTAAGCAAAATTC	17	121
5'-GGAGGCGGCGGTTCTGAGARTGTGGRGCWGCATC	8	122
5'-GGAGGCGGCGGTTCTAAKGARGTGGMGCAGRRTYC	2,15	123
5'-GGAGGCGGCGGTTCTCAGCTGCTGGAGCAGAG	10	124
5'-GGAGGCGGCGGTTCTCAAMAGRKAGAASAGRATYC	3,5	125
5'-GGAGGCGGCGGTTCTGGACAAARCMTTGARCAG	7	126
5'-GGAGGCGGCGGTTCTAAGGACCAAGTGTTTCAG	11	127

TCR-Vα BACK:

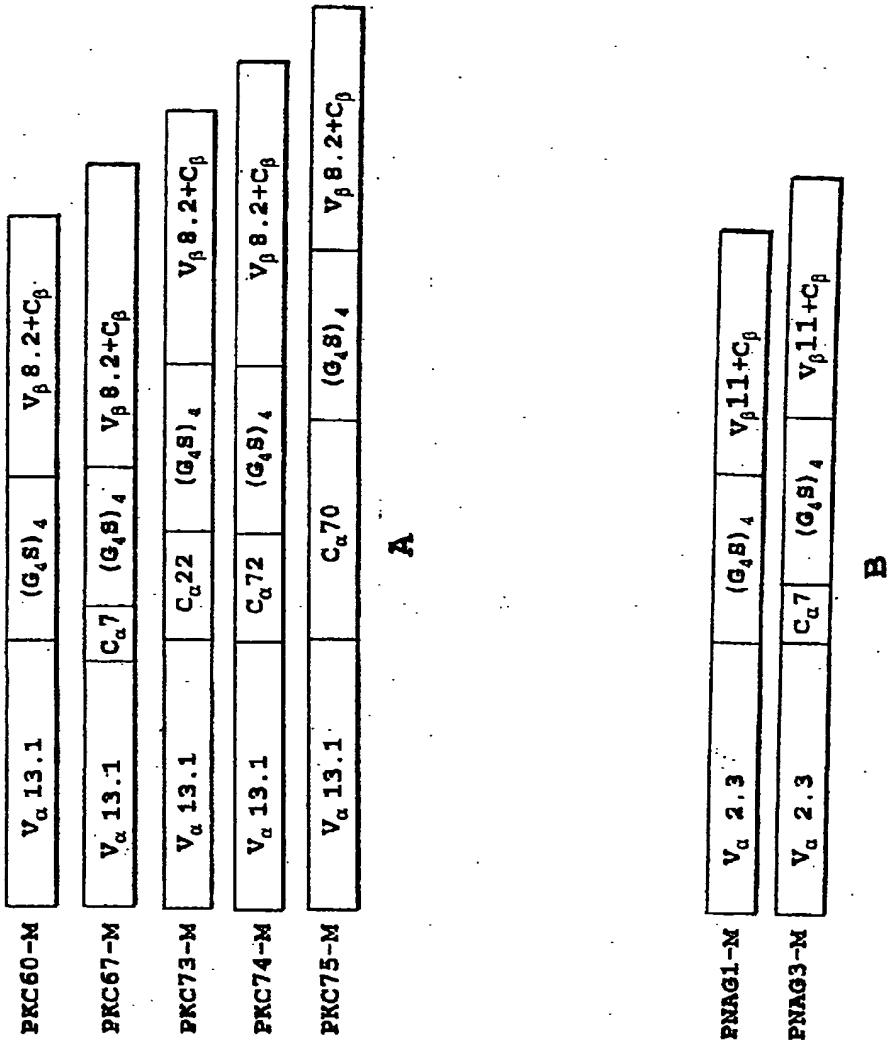
5'-TTCATAGACTAGTAGGGTCAGGGTTCTGG (SpeI) 128

(GGGGS) LINKER:

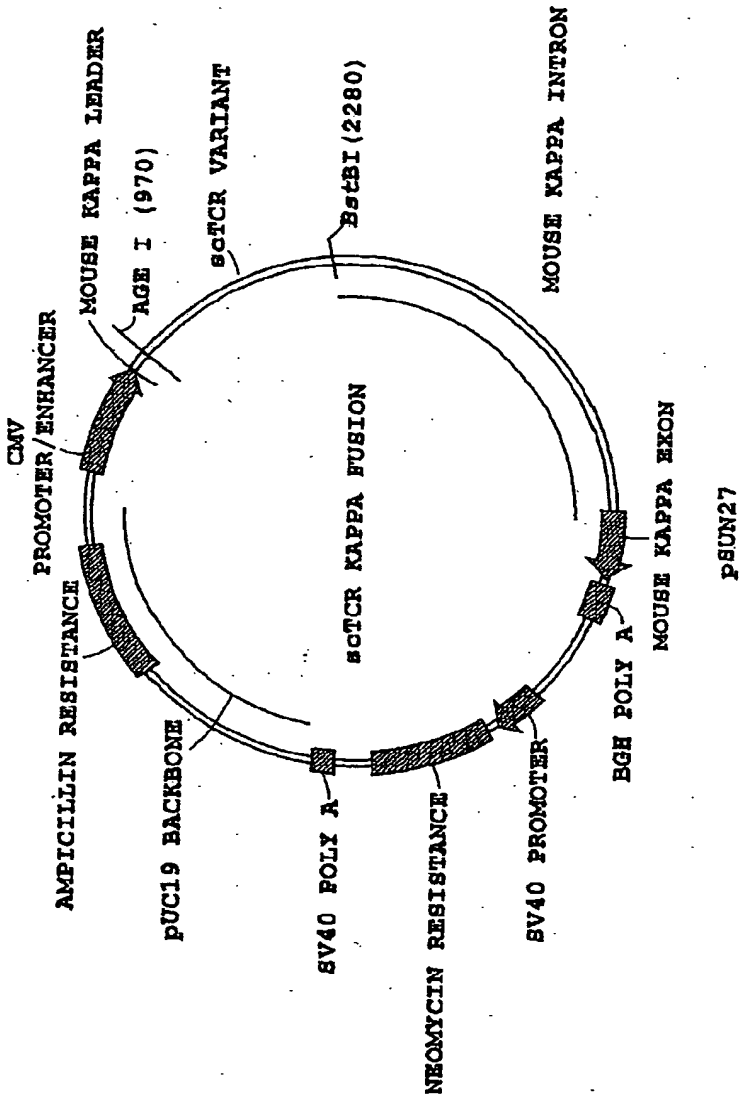
5'-GGTGGCGGTGGCAGCGGCGGTGGTGGTTCCGGAGGCGGCGGTTCT-3' 129

3'-CCACCGCCACCGTCGCCGCCACCACCAAGGCCTCCGCCGCCAAGA-5' 130

【図16】


PKC60-M
Vα 13.1
(G4S)4
Vβ 8.2+Cβ
PKC67-M
Vα 13.1
Cα7
(G4S)4
Vβ 8.2+Cβ
PKC73-M
Vα 13.1
Cα22
(G4S)4
Vβ 8.2+Cβ
PKC74-M
Vα 13.1
Cα72
(G4S)4
Vβ 8.2+Cβ
PKC75-M
Vα 13.1
Cα70
(G4S)4
Vβ 8.2+Cβ
A
PNAG1-M
Vα 2.3
(G4S)4
Vβ11+Cβ
PNAG3-M
Vα 2.3
Cα7
(G4S)4
Vβ11+Cβ
B

【図17】


CMV
PROMOTER/ENHANCER
MOUSE KAPPA LEADER
AGE I (970)
scTCR VARIANT
BstBI(2280)
MOUSE KAPPA INTRON
MOUSE KAPPA EXON
pSUN27
BGH POLY A
SV40 PROMOTER
NEOMYCIN RESISTANCE
SV40 POLY A
pUC19 BACKBONE
AMPICILLIN RESISTANCE
scTCR KAPPA FUSION

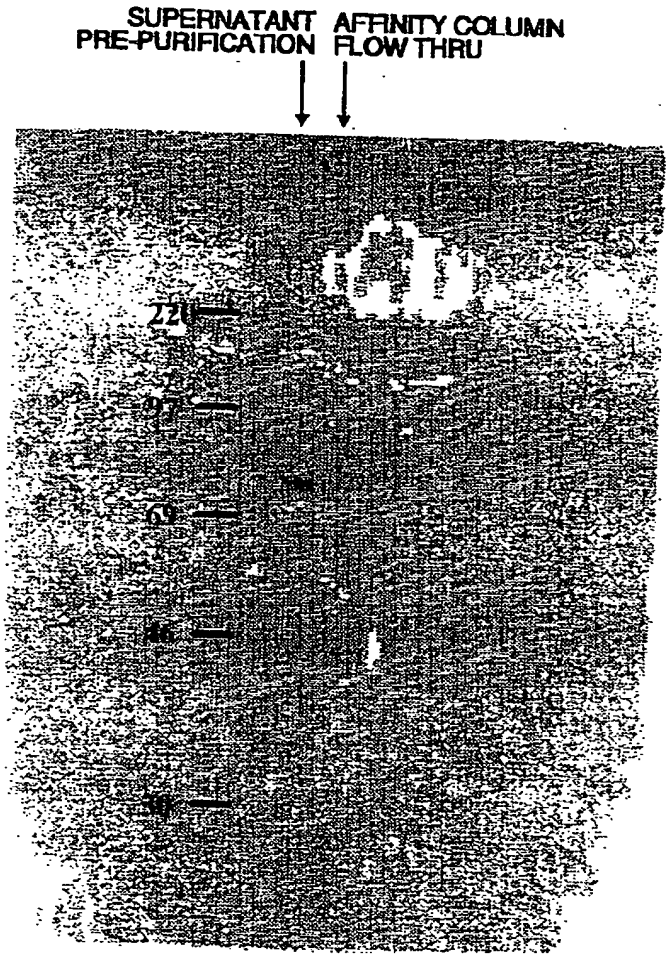
【図18】

		SEQ. ID. NO.
JWTCR208	gAg gTg gAA TTC TCA CCC ggg TTC CAT Cgg CAT gTA CTC TTC TTC CTC gTC TgC Tcg gCC CCA	75
JWTCR222	gAg CAC ggC CCA gCC ggC CAT ggC CgA ggC TgC AgT CAC CC	60
KC114	CTC gCg gCC CAg CCg gCC ATg gCC gAg CAg gTg gAg CAg CTT CCT	7
KC117	CTC gCg ACT AgT ACA ggg AAC gTC TgA ACT ggg	10
KC165	gAg gAg gTg gTg ACT AgT gCT gAg ggT gCT gCT CTg Ag	131
KC166	gAg gAg gTg gTg ACT AgT gCT ggT gAA gCT TgT CTg g	132
KC167	gAg gAg gTg gTg ACT AgT ACT ggg AAC gTC TgA ACT ggg	133
KC171	gAg gTg gAg gCC Cag Ccg gCC Atg gCC Cag gTg AgA CAA Ag	134
KC172	gAg gTg gAg CTC gAg CAA TgC Tgg TgT CAT CCA AAC	135
KC173	gAg gTg gAg ACT AgT gTC Aag gTT gAC CTg Aag	136
KC174	gAg gTg gAg ACT AgT AgC Agg TTC Tgg gTT CTg	137
KC176	gAg gTg gAg CCC ggg gTC TgC TCg gCC CCA ggC	138
KC199	g gCC Cag Ccg gCC AAT gCT ggT gTC ATC CAA AC	139
KC203	gAg gTg ACC ggT CAg CAg gTg AgA CAA AgT CC	140
KC204	gTg gAg ATC gAT AAag TgT ACT TAC gTT T gTC TgC TCg gCC CCA g	141

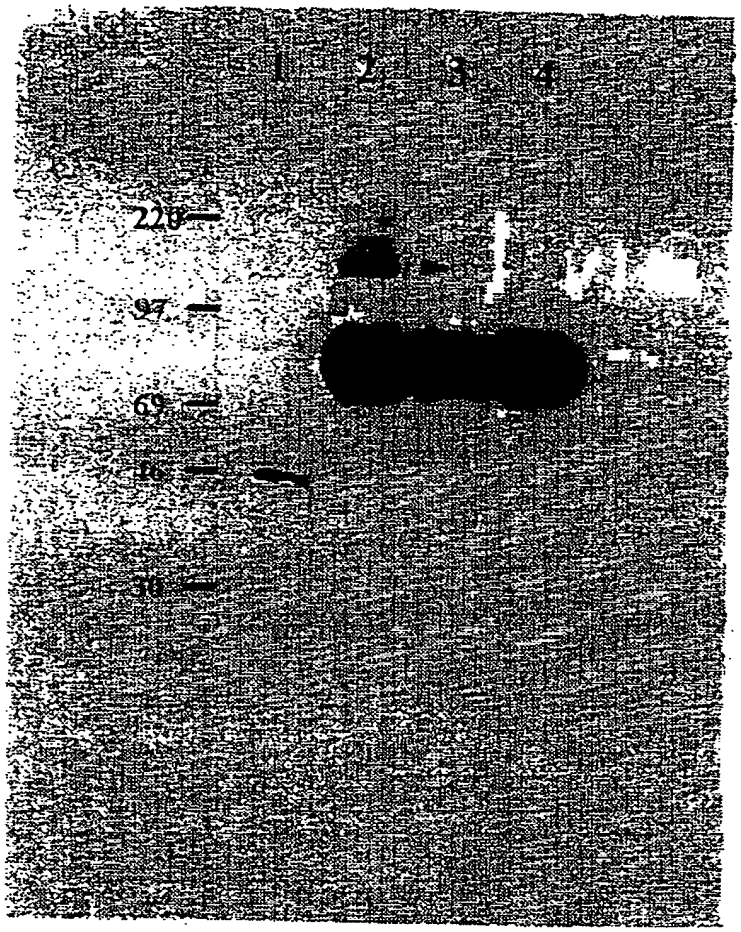
【図19】

		SEQ. ID. NO.
KC205	gTg gAg ATC gAT Aag TgT ACT TAC gTT T TCA TTA ACA gTC TgC TCg gCC CCA g	142
KC169	gAg gTg ACC ggT gAg CAg gTg gAg Cag CTT CC	143
KC170	gTg gAg TTC gAA Aag TgT ACT TAC gTT TgT CTg CTCg gCC CCA	144
KC201	gTg gAg TTC gAA AAg TgT ACT TAC gTTT TCA TTA gTC TgC TCg gCC CCA	145

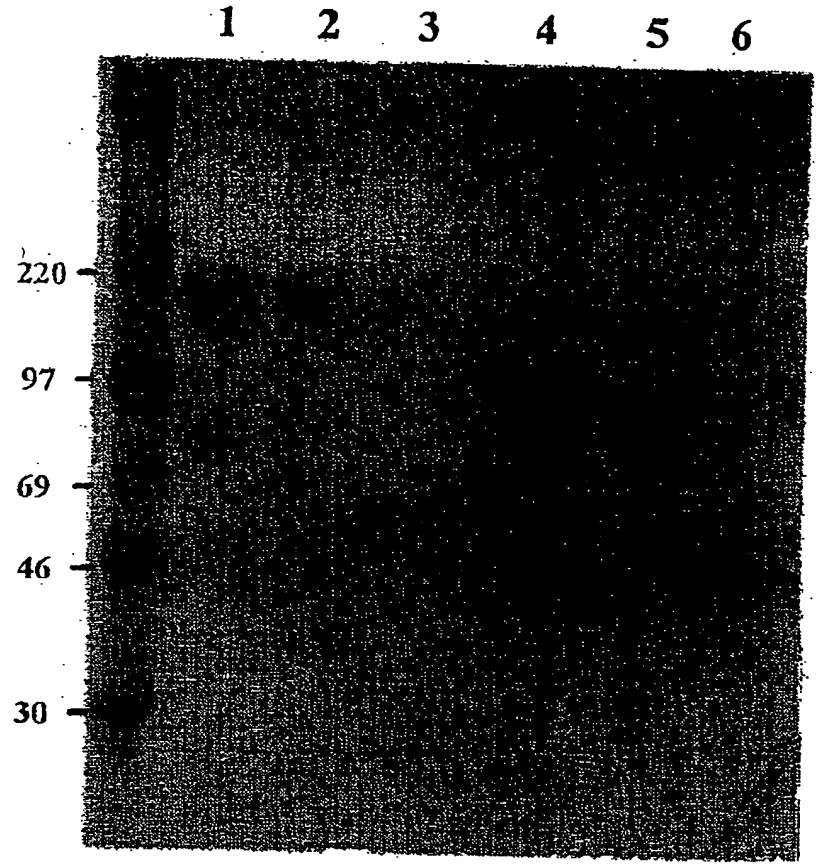
【図20】


SUPERNATANT
PRE-PURIFICATION
AFFINITY COLUMN
FLOW THRU
220
97
69
46
30

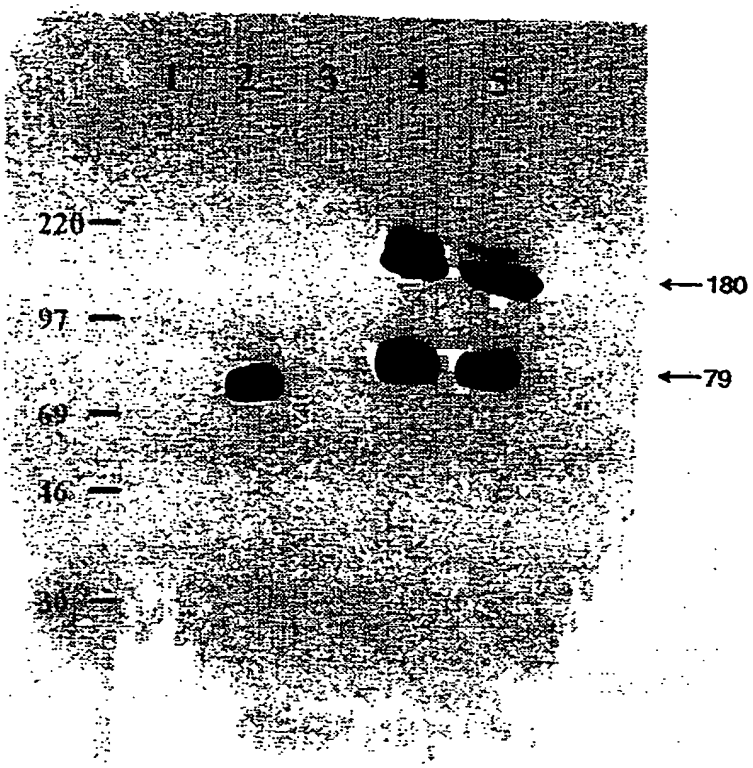
【図２１】



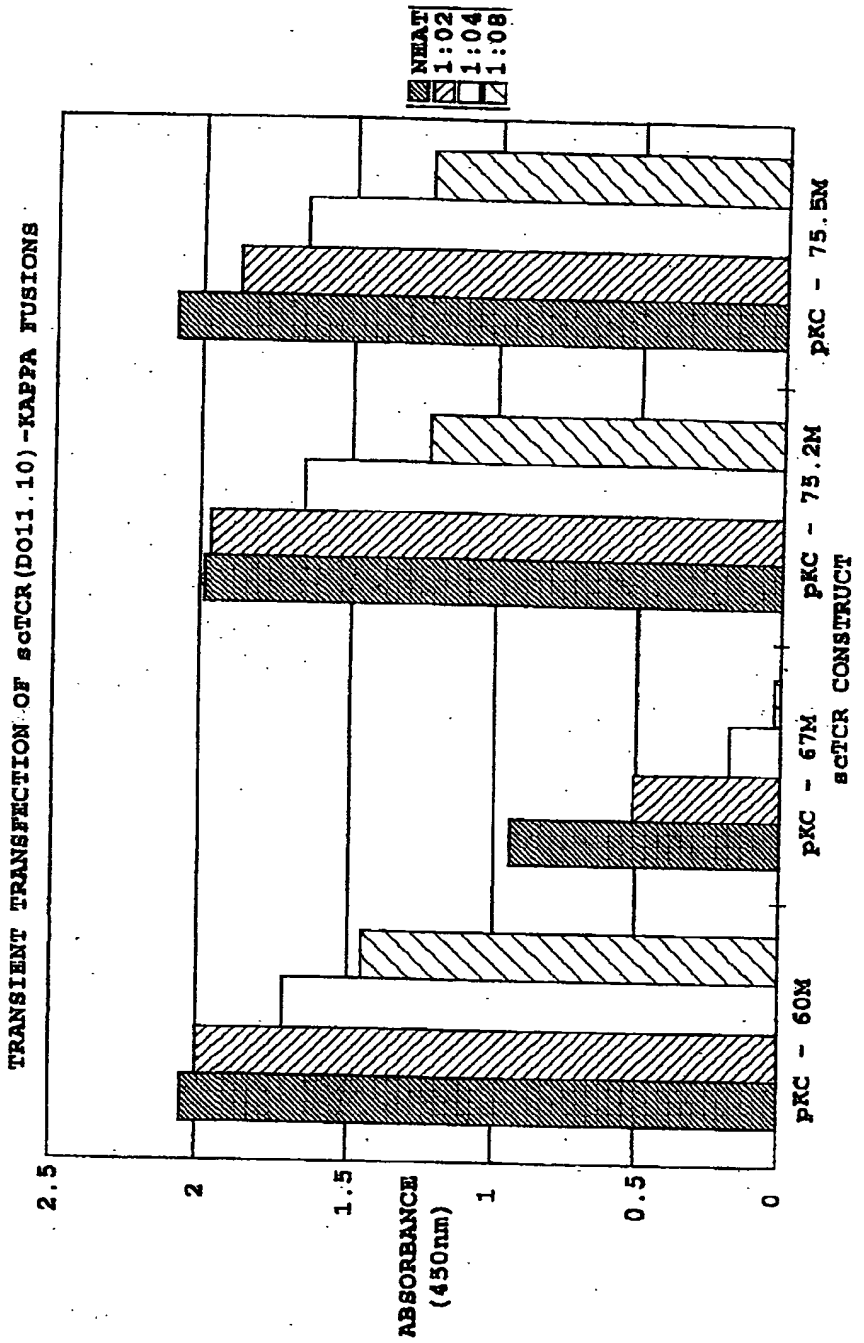
【図22】



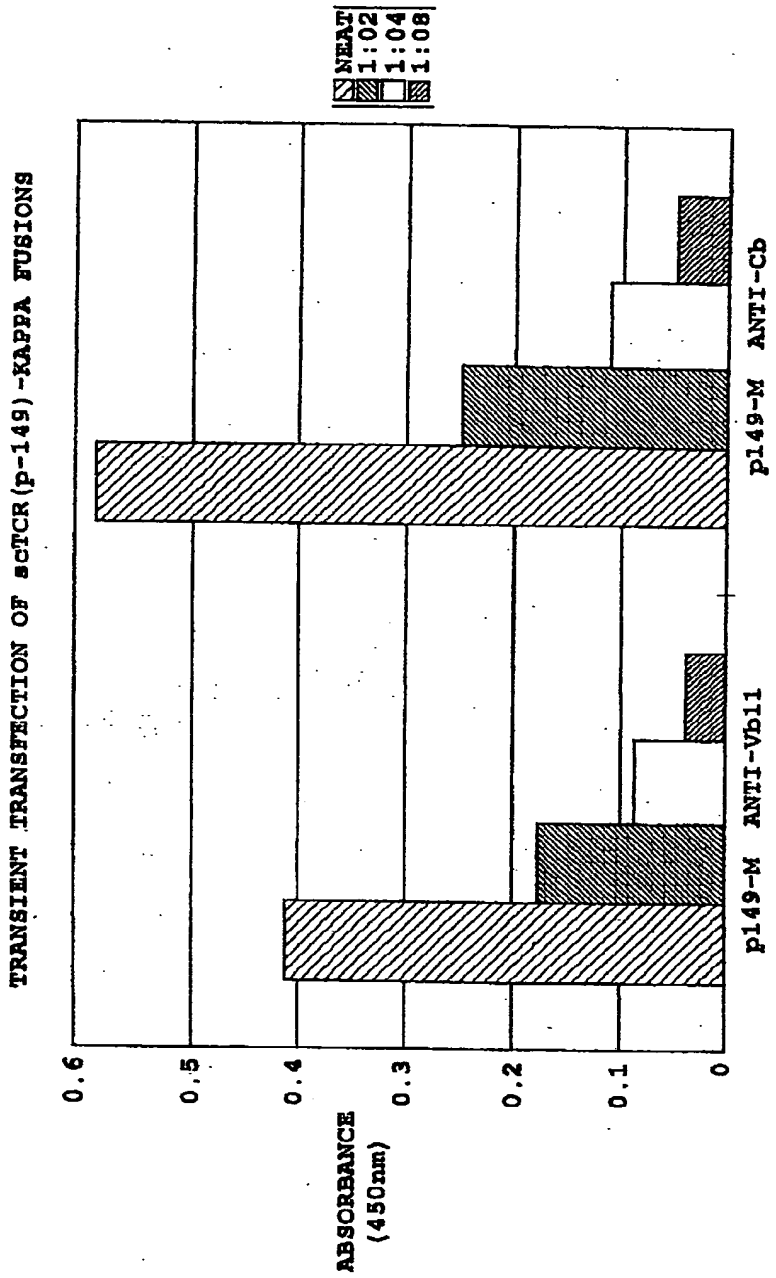
【図23】



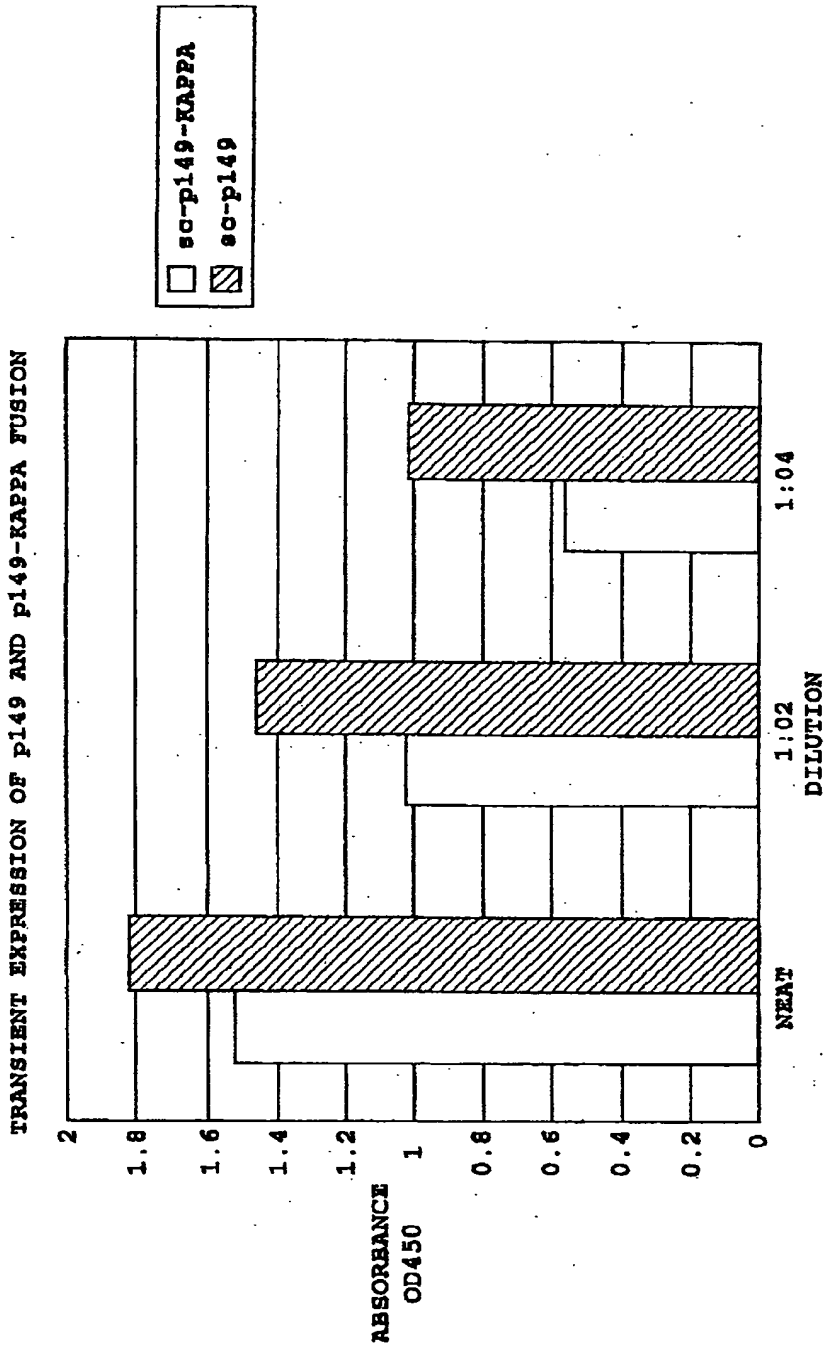
【図24】


TRANSIENT TRANSFECTION OF scTCR(DO11.10)-KAPPA FUSIONS
NEAT
1:02
1:04
1:08
ABSORBANCE (450nm)
2.5
2
1.5
1
0.5
0
pKC - 60M
pKC - 67M
pKC - 75.2M
pKC - 75.5M
scTCR CONSTRUCT

【図25】


TRANSIENT TRANSFECTION OF scTCR(p-149)-KAPPA FUSIONS
NEAT
1:02
1:04
1:08
p149-M ANTI-Cb
p149-M ANTI-Vb11
0.6
0.5
0.4
0.3
0.2
0.1
0
ABSORBANCE
(450nm)

【図26】


TRANSIENT EXPRESSION OF p149 AND p149-KAPPA FUSION
sa-p149-KAPPA
sa-p149
ABSORBANCE OD450
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NEAT
1:02
1:04
DILUTION

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/20263

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07K 14/725; A61K 38/00, 39/00
US CL :530/350, 395, 403; 424/185.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/350, 395, 403; 424/185.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Examiners T cell receptor files.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/02629 A1 (NATIONAL JEWISH CENTER FOR IMMUNOLOGY AND RESPIRATORY MEDICINE) 20 February 1992, see entire description as directed to production of chimeric TCR proteins and their uses.	1, 2, 4-15, 17, 21, 22, 35, 36, 39, 40
Y	US 5,565,335 A (CAPON ET AL.) 15 October 1996, see entire description as directed to production and usage of polypeptides lacking transmembrane domains.	1, 2, 4-15, 17, 21, 22, 35, 36, 39, 40

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 DECEMBER 1998	25 JAN 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer THOMAS CUNNINGHAM Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

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Total (1)	24	24	0	-

【図1】

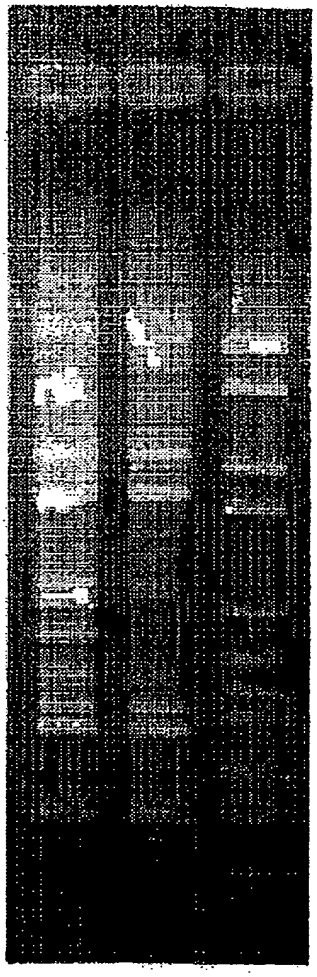

FIG. 1

【図2】

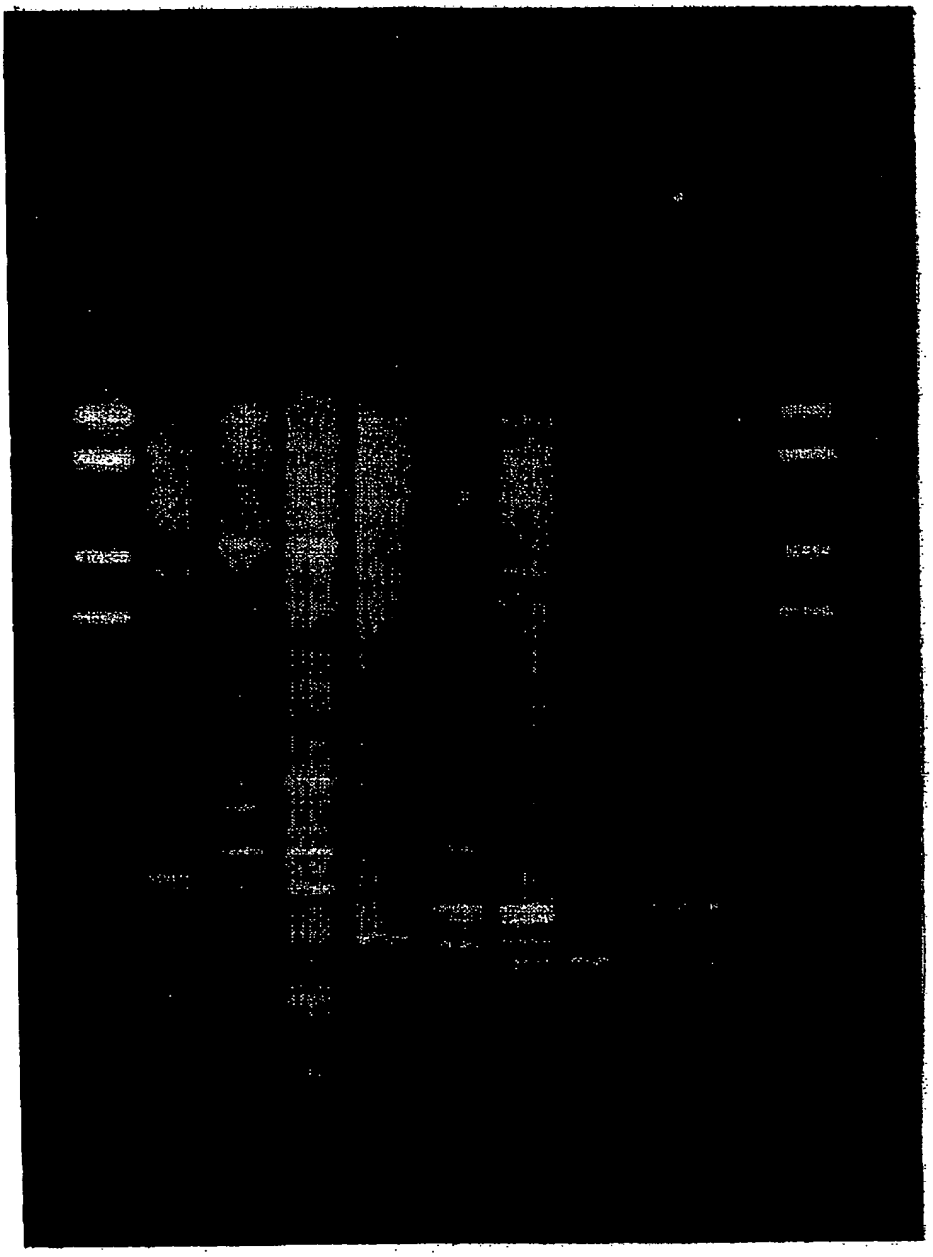

FIG. 2

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Total (1)	2	2	0	-

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Document Listing

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US005914331	24	1 - 24	1
Total (1)	24	-	-

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Summary

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Total (1)	2	2	0	-